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Effects of gel volume on pharmacokinetics for vaginal and rectal applications of combination DuoGel-IQB4012, a dual chamber-dual drug HIV microbicide gel, in pigtailed macaques

Lara E. Pereira[1], **Tyana Singletary**[2], **Amy Martin**[3], **Chuong T. Dinh**[4], **Frank Deyounks**[4], **Angela Holder**[3], **Janet McNicholl**[3], **Karen W. Buckheit**[5], **Robert W. Buckheit Jr.**[5], **Anthony Ham**[5], **David F. Katz**[6], **James M. Smith**[3]

[1]LifeSource Biomedical LLC, Moffett Field, CA, USA

[2]Anyar Inc, Fort Walton Beach, FL, USA

[3]Centers for Disease Control and Prevention, Division of HIV/AIDS Prevention, Atlanta, GA, USA

[4]Libra Management Group, Decatur, Georgia

[5]ImQuest BioSciences, Frederick, MD, USA

[6]Department of Biomedical Engineering, Duke University, Durham, NC, USA

Abstract

This study evaluated effects of differing gel volumes on pharmacokinetics (PK). IQB4012, a gel containing the non-nucleoside reverse transcriptase inhibitor IQP-0528 and tenofovir (TFV), was applied to the pigtailed macaque vagina and rectum. Vaginal gel volumes (1% loading of both drugs) were 0.5 or 1.5 ml; following wash-out, 1 or 4 ml of gel were then applied rectally. Blood, vaginal, and rectal fluids were collected at 0, 2, 4, and 24 h. Vaginal and rectal tissue biopsies were collected at 4 and 24 h. There were no statistically significant differences in concentrations for either drug between gel volumes within compartments at matched time points. After vaginal gel application, median IQP-0528 concentrations were ~ 10^4–10^5 ng/g, 10^5–10^6 ng/ml, and 10^3–10^5 ng/ml in vaginal tissues, vaginal fluids, and rectal fluids, respectively (over 24 h). Median vaginal TFV concentrations were 1–2 logs lower than IQP-0528 levels at matched time points. After rectal gel application, median IQP-0528 and TFV concentrations in rectal fluids were ~ 10^3–10^5 ng/ml and ~ 10^2–10^3 ng/ml, respectively. Concentrations of both drugs sampled in rectal tissues were low (~ 10^1–10^3 ng/g). For 1 ml gel, half of sampled rectal tissues had undetectable concentrations of either drug, and over half of sampled rectal fluids had undetectable TFV concentrations. These results indicate differences in drug delivery between the vaginal and rectal compartments, and that

James M. Smith ajo9@cdc.gov.

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smaller vaginal gel volumes may not significantly compromise microbicide PK and prophylactic potential. However, effects of rectal gel volume on PK for both drugs were less definitive.

Keywords

HIV prevention; Vaginal gel; Rectal gel; Macaque; Pharmacokinetics; IQP0528; PrEP

Introduction

Among the HIV preexposure prophylaxis (PrEP) modalities in development today, microbicide gels remain a significant arm of the antiretroviral (ARV)-based prevention pipeline. Although oral PrEP in the form of once-daily Truvada® (emtricitabine and tenofovir disoproxil fumarate) is now FDA approved in the USA [1] and has been shown to be effective at preventing HIV acquisition by 44–86% among various demographics [2–8] particularly when adherence is high, other biomedical prevention tools are also being developed to expand affordable and reliable options for at-risk populations. Alternative PrEP methods such as 30–90-day intravaginal rings, long-acting injectables, and on-demand products containing one or more ARVs are under research and development, to broaden users' choices and to help circumvent issues related to product adherence, potential long-term toxicity, accessibility, and/or cost concerns that can be associated with oral PrEP [4, 9–13].

Microbicide gels are intended to be an on-demand option for men and women who engage in vaginal and/or receptive anal intercourse (RAI). By coating the vaginal and rectal mucosa, they deliver drug directly down into the tissue, through which it migrates to infectible host cells along the length of the vaginal and rectal canals that may be exposed to semen [14]. They may also serve as physical barriers to HIV migration into the tissue [15, 16]. However, despite extensive studies of the vaginal gels, research on rectal gels has proceeded at a slower rate, and safe and efficacious rectal microbicide gels are still in the early stages of development [17, 18]. Extension and application of our knowledge of vaginal gels to rectal microbicide gels must address biological and behavioral challenges. Hyperosmolar vaginal gel formulations or personal lubricants are deemed unsuitable for rectal use due to epithelial damage and potentially increased risk of infection [19–21]. Vaginal gels as a PrEP modality in women have had low efficacy rates to date, for multiple reasons [4, 22–27]. Recent findings point to vaginal microflora as a contributing factor, with potential effects on genital inflammation, and ARV uptake and/or stability in vaginal tissues after gel application, but further studies of this phenomenon are needed [28–31]. A pattern that does clearly emerge from these clinical trials is behavior-based: low adherence is cited as a common factor across trials [4, 22, 25, 26, 32]. In light of these findings, emphasis is increasingly placed on participants' personal experiences and preferences in an effort to understand and overcome barriers associated with poor adherence. Studies have revealed product-related issues such as gel leakage and messiness, as well as undesirable effects on intercourse [32–38]. Recommended microbicide gel volumes for vaginal application have been typically 4–5 ml [4, 7, 22, 25, 26], and acceptability studies involving rectal gels have evaluated volumes ranging from 1.5 to 35 ml [39–44]. While smaller gel volumes might address some user

concerns regarding gel leakage, it is unclear whether these will achieve adequate gel distribution within the vaginal canal, and efficacious drug levels in vivo to protect against HIV infection. However, mathematical models evaluating the effects of gel volume on vaginal spreading and consequent mucosal drug delivery have predicted that smaller volumes may provide nearly equivalent drug delivery while reducing gel leakage [14].

While modeling and imaging studies are valuable for guiding microbicide gel design and use, the experimental in vivo pharmacokinetic (PK) implications of variable gel volumes have not previously been evaluated. Using reduced gel volumes is also motivated by the prior behavioral studies. We have previously described PK profiles of a dual chamber gel, IQB3002, which contains 1% of the NNRTI IQP-0528, a small molecule pyrimidinedione that inhibits a number of HIV strains at low EC_{50} concentrations [45, 46]. The gels' properties, particularly its low osmolality, are specifically formulated for safe application in both the vaginal and rectal compartments [47, 48]. Preliminary study of vaginal application in female rhesus macaques showed that IQP-0528 concentrations achieved in vaginal tissues and fluids were several orders of magnitude above the in vitro EC_{50} value [45]. Ex vivo virus infection assays further demonstrated that human peripheral blood mononuclear cells (PBMC) were protected from HIV-1 challenge when co-cultured with vaginal tissue biopsies from the gel-treated macaques [45]. Importantly, this level of protection was achieved with a reduced vaginal dose of 1.5 ml. That volume was allometrically scaled down versus a typical 4-ml clinically applied volume in humans via the ratio of approximate surface area of the rhesus macaque vagina to that in women. Collectively, our findings prompted further investigation of effects of gel volume in microbicide PK evaluations, for both rectal and vaginal gels.

The current study extends our previous work and evaluates PK following application of four different volumes of a related IQP-0528 gel, IQB4012, in female pigtailed macaques (PT). Two volumes (0.5 or 1.5 ml) were tested in the vaginal compartment and two volumes (1 or 4 ml) were applied in the rectum. Gel IQB4012 is similar to IQB3002 evaluated in our previous study in that it is formulated for dual application in the vagina and rectum (Table 1). In addition to 1% IQP-0528, IQB4012 also contains 1% of the NRTI tenofovir (TFV).

Materials and methods

Drug and product formulation

IQB4012 was manufactured by ImQuest BioSciences (Frederick, MD). The gel (Table 1) contains 1% (*w*/w) IQP-0528 [49–51], 1% (*w*/w) TFV, 10 mM sodium phosphate solution in sterile molecular grade water (93.15% *w*/w), glycerol (2.5% *w*/w), methylparaben (0.2% *w*/w), propyl paraben (0.05% *w*/w), hydroxyethyl cellulose (1% *w*/w), carbopol (1% *w*/w), EDTA (0.05% *w*/w), DL-lactic acid (0.05% *w*/w) [48]. It has a pH of 6.00, osmolality of 360 mOsm/kg and viscosity @ 1 s^{-1} = 128.7 Pa s. The release properties of IQP-0528 and TFV from the gel into a liquid sink (Franz Cell) are 53.93 and 82.51 μg/cm ^{3}h, respectively, with tissue permeability of 118.36 and 206.86 μg/cm^3, respectively. The product was stored at room temperature (25 °C) in a dark, moisture-free environment until use.

Pigtailed macaques and study design

All procedures in this study were approved by the Centers for Disease Control and Prevention IACUC (Institutional Animal Care and Use Committee). Animals were housed under approved biosafety level 2 containment conditions at the Centers for Disease Control and Prevention (CDC), and their diet, care, and maintenance conformed to "Guide for the Care and Use of Laboratory Animals" guidelines [52]. Six sexually mature, naive female pigtailed macaques (PT) with an average body mass of 8.7 kg were utilized in this study. All PT were anesthetized prior to procedures using 10 mg/kg ketamine mixture intramuscularly, or other approved anesthetic such as Telazol, 2–6 mg/kg intramuscularly, as determined by CDC Animal Resources Branch (ARB) standard operating protocol and ARB staff including the attending veterinarian. Ketamine and Telazol were supplied by the staff pharmacist in the Animal Resources Branch in accordance with CDC guidelines. For vaginal PK experiments, each PT received 0.5 ml or 1.5 ml of IQB4012 gel following anesthesia. Rationale for dosing (gel volume) was based on relative scaling of the size of the macaque female reproductive tract relative to humans. The upper volume is therefore equivalent by volume to a human dose of 4 mL. Gel was delivered into the posterior vagina, near the cervix, using a sterile 10-ml syringe attached to a sterile gastric feeding tube (size, 5 or 9 French; 7 to 8 cm in length). Syringes were weighed pre and post gel application to verify weight and volume of gel delivered. Animals were maintained recumbent under anesthesia with the pelvis slightly elevated for 30 min to minimize gel leakage. Animals were placed in two groups of three each. One group received 0.5 mL of IQB4012, while the other group received 1.5 mL IQB4012 once weekly for 4 consecutive weeks. After a 2-week rest period, the doses of the groups were then switched for another 4 weeks so that all six macaques received each dose four times. The data presented are therefore a summary of 24 applications of 0.5 and 1.5 mL of IQB4012.

For rectal PK experiments, a similar syringe apparatus was used to deliver 1 or 4 ml of IQB4012 into the rectum 2 in. past the sphincter of each PT after performing a rectal wash with 10 ml physiological saline. As with the vaginal PK study, the same crossover design was used with rectal gel application performed once weekly for 4 weeks with a 2-week rest period before the crossover and an additional 4 weeks of application. The rectal PK experiments were initiated several weeks after the vaginal PK study, to allow for a suitable wash-out period.

Specimen collections

For the vaginal gel application experiments, specimens collected for PK assessments included blood plasma, vaginal and rectal 3-mm punch biopsy specimens ($n = 3$ punch biopsies per site), vaginal secretions (multiswab device fitted with Weck-Cel® sponges, collected proximally and distally relative to the cervix), and rectal secretions approximately 5 cm into the rectum (Weck-Cel® spears). Spears and sponges were weighed before and after specimen collection. Vaginal pH was determined by collecting a swab of vaginal fluid and rolling it on a pH colorimetric indicator strip (EMD Millipore). Blood and swabs were collected immediately prior to (0 h) and at specific time intervals (2 h, 4 h, and 24 h) after IQB4012 application (Table 2). Tissue biopsies were collected at 4 h and 24 h only. Identical time points and specimens were used for collection of blood plasma, rectal fluids, and rectal

biopsies following rectal application of IQB4012 (Table 2). Blood was obtained via the saphenous or femoral veins using a 21–22 gauge vacutainer needle set-up into cell preparatory tubes (CPTs) and processed to obtain plasma and peripheral blood mononuclear cells. All specimens were collected, processed, and stored at − 70 °C until drug analysis as detailed previously [45].

Measurement of IQP-0528 and TFV concentrations

Vaginal fluids in individual multiswab Weck-Cel® sponges (two proximal and two distal per animal per time point each week), and rectal fluids in individual Weck-Cel spears (two spears per animal per time point each week) were analyzed for IQP-0528 and TFV levels. Each week, biopsy samples that included three punches from vaginal tissues (one proximal, one medial, one distal per time point per animal), and two punches from rectal tissues collected approximately 5 cm into the rectum were analyzed per animal per time point. Unlike the active form of TFV, IQP-0528 is rapidly transported bi-directionally across the cell membrane and is not restricted within the cell, and so tissues were not washed prior to analysis to avoid drug loss [46]. For plasma, 100 μl aliquots per animal per time point from each week were analyzed. The concentrations of IQP-0528 and TFV in the biological samples were determined based on a previously described liquid chromatography-tandem mass spectrometry (LC-MS) method [45, 53, 54]. The minimum level at which quantitative results could be obtained, the lower limit of quantitation (LLOQ), is ten times the standard deviation of injections at the lowest concentration which were statistically different from blank injections using a 99% confidence interval. The LLOQ of IQP-0528 for tissues, vaginal or rectal fluids, and plasma were determined to be 10 ng/sample for biopsies and 10 ng/ml for fluids. Vaginal fluid and tissue densities of 1.0 g/ml were used to convert weight to volume concentrations of IQP-0528 and TFV (ng/g of fluid or tissue). Reported concentrations in vaginal secretions were corrected to account for sponge net weight and dilution factors. Data for rectal fluids are reported as ng/ml of spear eluate.

Statistical analyses

Data shown are medians with minimum and maximum values unless indicated otherwise. To analyze differences in IQP-0528 and TFV levels across multiple time points and animals, one-way analyses of variance (ANOVA) were applied, with Dunns post-test analysis. Significant paired data sets ($p < 0.05$) were further analyzed ad-hoc using the Wilcoxon matched-pairs signed-rank t test. Analyses were performed using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA).

Results

Vaginal application of IQB4012

Specimens collected over a 24-h period after 0.5 ml gel application showed that median IQP-0528 concentrations in vaginal tissues and vaginal fluids at 4 h (open black boxes, Fig. 1a, b; Table 3) were of the order of 10^5 ng/g (95,000–290,086 ng/g) and 10^6 ng/ml (2,038,462−6,758,116 ng/ml), respectively. These were followed by a statistically significant ($p < 0.0001$) one log decrease in vaginal fluids at 24 h (257,071–328,205 ng/ml). IQP-0528 levels in vaginal fluids at 2 h were similar to those observed at 4 h. The graphs (Fig. 1a, b)

illustrate pooled data from proximal, medial, and distal sites relative to the cervix, with Table 3 listing median concentrations at each of those sites. Data were grouped for analysis (Fig. 1a, b) as there was no significant difference in IQP-0528 concentrations across those sites at matched time points. Interestingly, application of 1.5 ml gel (open gray boxes, Fig. 1a, b; Table 3) yielded similar results in vaginal tissues and fluids, with IQP-0528 concentrations being in the same range as that following 0.5 ml gel treatment (open black boxes, Fig. 1a, b; Table 3). Slight amounts of gel were observed to be present at the 2-h collection, but gel was not present in the vast majority of the 4-h samples. Low concentrations of IQP-0528 were detected in rectal fluids (Fig. 1c and Table 3) following vaginal application of either volume of IQB4012, with median levels being in the range of 10^1–10^3 ng/ml over 24 h (20–1258 ng/ml). A significant difference between the two gel volumes was noted for IQP-0528 concentrations in rectal fluids at 2 h (open black and open gray boxes, Fig. 1c), but not at later time points. Analysis of rectal tissue biopsies revealed IQP-0528 levels below LLOQ (data not shown).

Similar to the trends observed for IQP-0528 PK, there were no significant differences in TFV concentrations across all time points when comparing applications of 0.5 and 1.5 ml gel in the vaginal canal. Median levels of TFV were 1–2 logs lower than IQP-0528 concentrations at matched time points over the 24-h period. They were 10^3–10^4 ng/g in vaginal tissues (4486–13,367 ng/g for 0.5 ml gel; 10,181–35,081 ng/g for 1.5 ml gel) and 10^4–10^5 ng/ml in vaginal secretions (124,304–132,458 ng/ml for 0.5 ml gel; 54,269–152,548 ng/ml for 1.5 ml gel) at 4 h, followed by a negligible decrease in concentrations at 24 h (striped black and striped gray boxes, Fig. 1a, b; Table 3). Similar to IQP-0528 levels in the rectum, median concentrations of TFV in rectal secretions were low and not always detectable and did not exceed 200 ng/ml over 24 h (striped black and striped gray boxes, Fig. 1c; Table 3). Concentrations in rectal biopsy tissues were below the LLOQ (1 ng/sample, data not shown). In summary, these data collectively show that a twofold difference in gel volume (0.5 versus 1.5 ml) did not have a significant impact on the PK of either IQP-0528 or TFV in the vaginal compartment. Plasma concentrations of both compounds were below the LLOQ (10 ng/ml, data not shown).

Rectal application of IQB4012

After a wash-out period following the vaginal PK study, rectal PK experiments were conducted in the same macaques. Either 1 or 4 ml of IQB4012 gel was applied rectally. Rectal spears were collected at 2, 4, and 24 h, and rectal pinch biopsies were collected at 4 and 24 h, which are time points identical to those considered in the vaginal PK study. Application of 1 ml IQB4012 gel yielded median IQP-0528 concentrations in the two-log range, 10^3–10^5 ng/ml in rectal fluids. There was a statistically significant ($p < 0.0001$) decrease from 93,375 ng/ml at 2 h to 2975 ng/ml at 24 h (open black bars, Fig. 2a; Table 4). Following application of 4 ml of gel, similar IQP-0528 concentrations were observed in rectal fluids over the 24-h period (open gray bars, Fig. 2a; Table 4). Interestingly, despite the relatively high IQP-0528 concentrations present in rectal fluids, approximately half of the rectal tissue samples analyzed were found to have IQP-0528 concentrations below the LLOQ. This was primarily found in the low volume group, where nearly two-thirds of rectal biopsies from animals treated with 1 ml had undetectable levels of IQP-0528. Tissue

samples with detectable concentrations were in the range of 10^2–10^3 ng/g for both gel volumes tested (black open bars and gray open bars, Fig. 2b; Table 4), and there was no significant difference in IQP-0528 concentrations between the two gel volumes at matched time points.

Analysis of TFV levels in rectal fluids showed similar patterns to those for IQP-0528. Volumes of 1 ml (striped black bars, Fig. 2a; Table 4) and 4 ml (striped gray bars, Fig. 2a; Table 4) yielded comparable drug concentrations at matched time points. Specifically, median fluid TFV concentrations were on the order of 10^3 ng/ml (3230 ng/ml for 1 ml gel; 5065 ng/ml for 4 ml gel) at 2 h, and 10^2 ng/ml at 4 h (705 ng/ml for 1 ml gel; 864 ng/ml for 4 ml gel) and 24 h (132 ng/ml for 1 ml gel; 277 ng/ml for 4 ml gel). It should be noted that approximately two-thirds of rectal fluid samples from the low volume group yielded TFV levels below the LLOQ. The levels of TFV and TFV-diphosphate (TFV-DP), the active intracellular form of TFV, in approximately half of the rectal tissue samples from animals treated with 1 ml gel were also below the LLOQ, with detectable concentrations (Table 4) being at or near the magnitude of 10^2 ng/g at 4 h (811 ng/g TFV; 176 ng/g TFV-DP) and 24 h (659 ng/g TFV; 70 ng/g TFV-DP). Median concentrations of TFVand TFV-DP in rectal tissues from the 4-ml gel volume group (striped gray bars and dotted gray bars, Fig. 2b; Table 4) were not significantly different from the low volume group at matched time points. Median TFV and TFV-DP concentrations were near 10^2–10^3 ng/g or less at 4 h (393 ng/g TFV; 47 ng/g TFV-DP) and 24 h (1,411 ng/g TFV; 81 ng/g TFV-DP). To summarize, the PK results showed that IQP-0528 and TFV/TFV-DP concentrations in the rectum were each comparable between the 1 and 4 ml gel groups over a 24-h period, with a notable number of the rectal specimens yielding values below LLOQ (10 ng/sample), particularly for TFVand the low volume (1 ml) group. Systemic drug absorption was not apparent after rectal application of IQB4012, as levels of both drugs were below the LLOQ (10 ng/ml) in blood plasma (data not shown).

Discussion

Microbicide gel volume is one of many factors that influence user acceptability, but its impact on in vivo PK has not been fully elucidated. The impact of gel volume may differ among microbicide drugs with different physicochemical properties and may be different for vaginal versus rectal gel application. The present study addressed three factors; gel volume, drug and target compartment. The test gel, IQB4012, has been developed specifically to address the need for a safe and effective rectal microbicide product, particularly given the prevalence of receptive anal intercourse in heterosexual populations and its impact on HIV transmission [55–64]. Because IQB4012 is formulated for safe application both in the vagina and rectum [47, 48], convenience to user(s) is promoted, bypassing the need for separate products for vaginal and rectal use and potentially improving product acceptability and adherence. This formulation was engineered as an improvement on its predecessor, IQB3002, with rheological properties designed not simply to promote good initial spreading along the vaginal and rectal canals, but improved retention after initial application (Table 1).

MRI-based in vivo imaging studies of two volumes (2.5 versus 3.5 ml) of up to seven different microbicide gels and personal lubricants in women showed that there were no

statistically significant differences between the volumes tested [65]. Recently, an optical imaging study comparing vaginal applications of 2 versus 4 ml of the HEC gel used as a placebo in microbicide trials, found differences in coating thickness but not extent of coating along the canal; computational modeling inferences from those data suggested small differences in mucosal drug delivery between the two volumes [66]. However, it is important to note that besides volume and gel composition, host factors such as ambulation, presence of semen, and local dilution of gel (estimated to range 10–30%) due to ambient fluid in the vaginal compartment, also impact a gel's rheological properties and consequent deployment and drug delivery [67–71].

Co-formulation of IQB4012 with both IQP-0528 and TFV provides two drugs with contrasting physicochemical properties: IQB4012 is hydrophobic and lipophilic, while TFV is relatively hydrophilic. Those distinctions impact the relative transport rates of the drugs out from the gel and into and through the mucosal tissue. These two drugs have exhibited additive to synergistic activity that is concentration dependent when tested with primary HIV-1 isolates in vitro [72]. IQP-0528 diffuses bi-directionally across cell membranes, whereas TFV is phosphorylated within cells and retained with a long half-life [46, 73, 74]. This allows for one of the active drugs to be readily available to trafficking cells. These differing characteristics may well be advantageous for better protection when the two drugs are delivered together. The susceptible cells in the mucosa are loaded with TFV-DP, and this has been a good correlate of protection in macaque efficacy studies with gels and intravaginal rings [73, 75]. This formulation advantage may be similar to what has been observed for the combination of TFVand FTC in macaque efficacy studies where the PK of the two compounds lead to a more broad window of protective efficacy than a single drug alone [76].

Results from this study show that the concentrations of IQP-0528 measured in fluids from the vaginal compartment after vaginal application were 5–6 logs higher than the reported EC_{50}/IC_{50} value for IQP-0528 [46, 47, 49–51, 73, 77] up to 24 h post gel application, and were 1–3 logs higher than the EC_{50} in rectal fluids (Table 3, ratio of measured/EC_{50}). In contrast, the measured drug/EC_{50} ratio for TFV when applied vaginally was substantially lower than (range 26–1009) reflecting the difference in potency of the two drugs (Table 3). A very similar trend was evident when the gel was applied rectally (Table 3), with IQP-0528 ratios being 3–5 logs higher than the EC_{50} and the TFV ratios being substantially lower in rectal fluids (range 0.12–7.35).

Results showed no significant differences between applied gel volumes in fluid and tissue levels for both IQP-0528 and for TFV in the vagina and in the rectum. Further, rectal tissue TFV-DP levels did not differ between volumes when applied rectally. Differences were not detected in tissue concentrations of both drugs sampled at proximal versus medial versus distal locations along the vaginal canal, i.e., their longitudinal drug delivery was uniform. Furthermore, bi-directional dosing was observed as shown by presence of drug, albeit at low concentrations, in rectal fluids following vaginal application of both 0.5 and 1.5 ml IQB4012. Similar effects were observed when comparing PK data for the two volumes (1 versus 4 ml) tested in the rectal PK study, although the majority of specimens collected from the low volume (1 ml) group had undetectable levels of IQP-0528 and TFV. These results

were somewhat surprising given that the total drug in the lower volumes was one fourth of that in the higher volumes. However, the range of measured values was such that a fourfold difference would be difficult to detect.

We emphasize, however, that IQP-0528 (and other microbicides, more generally) delivery and potency within the rectal compartment may be different from those for the vagina. Many factors could contribute to this, including: compartment size and openness; composition of luminal fluids and luminal drug clearance processes; mucosal tissue structure and cell types, including target cells and their distributions; mucosal tissue permeability; and pH and the microbiome. In addition to differences in length and mucosal surface area, the mucosal structures of the two organs are different. The vagina has a multilayer, non-secretory, stratified squamous epithelium with thickness of hundreds of micrometers. Infectible host cells populate the lamina propria below it, which is primarily connective tissue. The rectal mucosa is characterized by a single layer of mucus-secreting columnar epithelial cells arranged in rugae that extend millimeters down into the mucosa; the lamina propria wraps around the rugae, and extends downward, containing pockets of lipids within its connective tissue. These qualitatively different environments present different permeabilities to migrating molecules, and virions. Vaginalrectal contrasts in TFV delivery have been predicted by a deterministic computational PK model [78].

The threshold for minimum volumes may be higher for the rectum than the vaginal canal because of physiological and anatomical differences. If potentially efficacious drug levels can be achieved with lower gel volumes as suggested by these results, a “less is more” approach presents a feasible option that could help improve user perceptibility and experience of this gel, thereby encouraging product adherence. However, user sensory perceptions are multifaceted and what might be acceptable to some may not be to others. The data reported herein are only intended to provide information about the viability of additional choices. Further investigation is needed, particularly to determine the optimal volume for use in the rectal compartment. There is currently no consensus for rectal microbicide gel volumes, unlike the relatively narrow range used for vaginal gels (3.5–4 ml in clinical trials); a wide range (1.5–35 ml) of volumes has been tested for rectal use thus far. Other gel formulation properties that govern spreading and absorption may have to be adjusted accordingly to account for volume, especially when user-dependent characteristics come into play. Indeed, a caveat of the current study is that it is not designed to factor in ambulation due to necessary sedation of macaques during experiments. Thus, its effect on gel spreading and subsequent PK relative to gel volume remains to be elucidated. In addition, we also cleansed the area with a 10-ml saline wash prior to insertion. Cleansing prior to RAI is common, but not always the case. The IQB4012 gel formulation has demonstrated good safety profiles in human ectocervical and colorectal tissue explants, while also effectively inhibiting HIV-1 replication in these tissue models at concentrations as low as 10 μM (A. Ham et al., unpublished data). The findings from the in vivo study described herein build upon these promising characteristics of IQB4012. They complement computational PK models and imaging studies and provide further insight on ARV PK in the context of gel volume. This, in turn, can inform microbicide gel applications in clinical trial settings.

In summary, our results collectively show that the dual compartment IQB4012 gel is capable of delivering both IQP-0528 and TFV in vaginal and rectal tissues to levels well above concentrations that are inhibitory to HIV-1 in vitro. Lowering gel volumes by a factor of 3–4, and a corresponding reduction of total drug, did not appear to significantly impact PK of IQP-0528 and TFVin the vaginal and rectal compartments. Additional studies using a reduction in total drug of tenfold or more might result in a significant difference in drug levels, but a reduction of tenfold in volume was beyond the scope of this study. Importantly, our findings are consistent with our previous report evaluating vaginal application of 1.5 ml IQB3002 gel formulation, and also align with optical imaging and computational modeling studies that showed pharmacologically small effects when varying gel volume [79]. These findings provide relevant information for clinical trials of microbicide gels, and lay a foundation for future studies that include investigating the impact of lower gel volumes on efficacy.

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All experiments comply with the current laws of the country in which they were performed. All institutional and national guidelines for the care and use of laboratory animals were followed.

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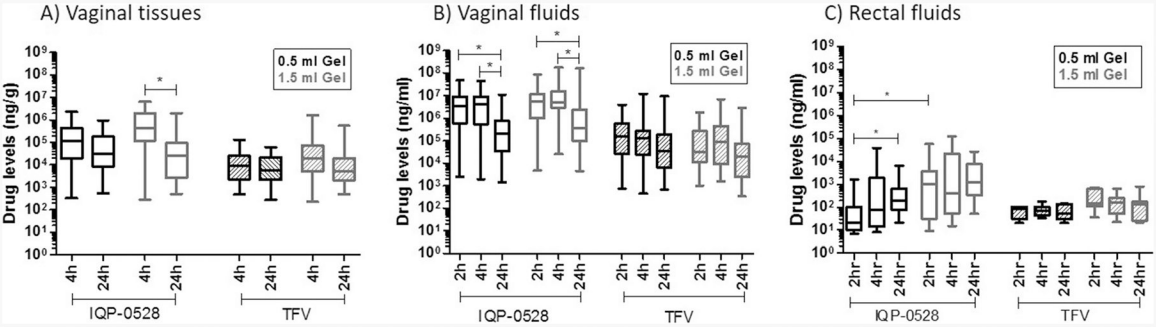

Fig. 1.

Analysis of IQP-0528 (open) and TFV (striped) levels in **a** vaginal tissues, **b** vaginal fluids, and **c** rectal fluids from $N = 6$ macaques treated vaginally with 0.5 (black) or 1.5 ml (gray) IQB-4012 gel. Results shown for vaginal samples include pooled data from proximal, medial, and distal sites. Concentrations at each of these sites are listed in Table 2. One-way ANOVA test with Dunns post-test analysis was performed. Significant paired data sets ($p < 0.05$) were further analyzed ad-hoc using Wilcoxon matched-pairs signed-rank t test, and yielded p values < 0.0001 where indicated (*)

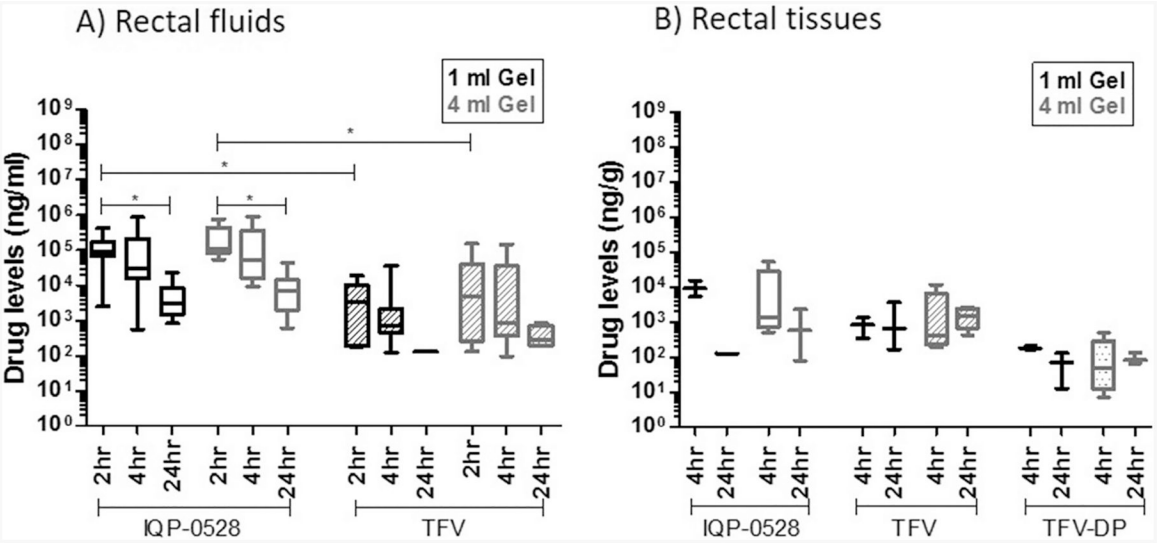

Fig. 2.

Analysis of IQP-0528 (open), TFV (striped), and TFV-DP (dotted) levels in **a** rectal fluids and **b** rectal tissues from $N = 6$ macaques following rectal application of 1 (black) or 4 ml (gray) IQB-4012 gel. Concentrations at each of these sites are detailed in Table 3. One-way ANOVA test with Dunns post-test analysis was performed. Significant paired data sets ($p < 0.05$) were further analyzed ad-hoc using Wilcoxon matched-pairs signed-rank *t* test, and yielded $EC_{50} < 0.0001$ where indicated (*)

Table 1

DuoGel formulations

Ingredient (*w*/w, %)	3002	4012
Tenofovir	/	1.00
IQP-0528	1.00	1.00
Phosphate buffer	93.90	93.15
Glycerin	2.50	2.50
Hydroxyethyl cellulose	2.10	1.00
Carbopol	0.25	1.00
Methylparaben	0.20	0.20
Propylparaben	0.05	0.05
EDTA	/	0.05
Lactic acid	/	0.05

Table 2

Outline of study design and specimen collections. For the vaginal PK study, female pigtailed macaques (N = 6) were treated with 0.5 or 1.5 ml IQB4012 that was applied in the posterior vagina, proximal to the cervix. For the rectal PK study, 1 or 4 ml IQB4012 was applied in the rectum of N = 6 female pigtailed macaques. Specimens indicated were collected immediately before (0 h) and at 2, 4, and 24 h after each weekly gel application

Sample	0 h*	2 h	4 h	24 h
Blood[a] (vaginal and rectal PK)	X	X	X	X
Vaginal multiswab[b] (vaginal PK)	X	X	X	X
Rectal spears[c] (vaginal and rectal PK)	X	X	X	X
Vaginal pinch biopsies[d] (vaginal PK)	-	-	X	X
Rectal pinch biopsies[e] (vaginal and rectal PK)	-	-	X	X
Vaginal pH[f] (vaginal PK)	X	X	X	X

[a] 2-ml CPT tubes

[b] Vaginal multiswab device loaded with eight Weck-Cel sponges; four proximal and four distal

[c] Two rectal wicks/animal/time point

[d] VAGINAL: three pinch biopsies/animal/time point (one prox, one med, one dist)

[e] RECTAL: two pinch biopsies/animal/time point

[f] Vaginal pH: pH indicator strips

Table 3

Drug concentrations in vaginal tissues, vaginal fluids, and rectal fluids from $N = 6$ female pigtailed macaques treated vaginally with 0.5 or 1.5 ml IQB4012 gel containing 1% wt/wt each of IQP-0528 and TFV

		IQP-0528 median drug concentration (min—max)					
		Vaginal tissues (ng/g)			**Vaginal fluids (ng/ml)**		**Rectal fluids (ng/ml)**
		Proximal[b]	**Medial**	**Distal**	**Proximal**	**Distal**	-
0.5 ml Gel	2 h	N/A	N/A	N/A	6,758,116 (2474–46,372,550)	2,319,091 (BLLOQ-17,500,000)	20 (BLLOQ-1583)
	4 h	128,443 (323–2,344,444)	290,086 (887–1,503,333)	95,000 (BLLOQ-1,839,7441	6,014,248 (1946–43,815,790)	2,038,462 (BLLOQ-17,891,890)	77 (BLLOQ-37,600)
	24 h	43,878 (1622–962,500)	22,527 (714–458,763)	20,915 (545–224,753)	257,071 (BLLOQ-5,200,000)	328,205 (BLLOQ-11,034,8801	199 (BLLOQ-6288)
	Ratio (measured/ EC_{50}[a])	N/A	N/A	N/A	2 h-6,758,116	2 h-2,319,091	2 h-20
					4 h-6,014,248	4 h-2,038,462	4 h-77
					24 h-257,071	24 h-328,205	24 h-199
1.5 ml Gel	2 h	N/A	N/A	N/A	5,654,526 (4714–57,865,860)	6,022,388 (BLLOQ-83,227,270)	1004 (BLLOQ-56,000)
	4 h	611,547 (449–4,760,736)	739,886 (278–6,377,273)	213,000 (BLLOQ-6,404,545)	4,925,373 (BLLOQ-60,750,000)	4,636,643 (BLLOQ-173,000,000)	411 (BLLOQ-120,000)
	24 h	23,873 (1489–1,054,000)	46,315 (1087–2,031,746)	24,167 (BLLOQ-517,742)	475,000 (BLLOQ-159,000,000)	375,385 (BLLOQ-32,714,2901	1258 (BLLOQ-26,475)
	Ratio (measured/ EC_{50})	N/A	N/A	N/A	2 h-5,654,526	2 h-6,022,388	2 h-1004
					4 h-4,925,373	4 h-4,636,643	4 h-411
					24 h-475,000	24 h-375,385	24 h-1258
		TFV median drug concentration (min—maxi					
		Vaginal tissues (ng/g)			Vaginal fluids (ng/ml)		Rectal fluids (ng/ml)
		Proximal	Medial	Distal	Proximal	Distal	-
0.5 ml Gel	2 h	N/A	N/A	N/A	158,146 (BLLOQ[2]-3,025,157)	232,128 (BLLOQ-3,968,750)	80 (BLLOQ-104)
	4 h	4486 (484–60,000)	8857 (714–130,909)	13,367 (BLLOQ-72,051)	124,304 (BLLOQ-12,113,400)	132,458 (BLLOQ-1,971,250)	69 (BLLOQ-176)

		IQP-0528 median drug concentration (min—max)					
		Vaginal tissues (ng/g)			Vaginal fluids (ng/ml)		Rectal fluids (ng/ml)
		Proximal[b]	Medial	Distal	Proximal	Distal	-
	24 h	16,932 (270–61,786)	3553 (BLLOQ-30,000)	5906 (BLLOQ-37,030)	48,942 (BLLOQ-9,600,000)	18,709 (BLLOQ-267,794)	53 (BLLOQ-139)
	Ratio (measured/ EC_{50})	N/A	N/A	N/A	2 h-230	2 h-1009	2 h-0.12
					4 h-180	4 h-192	4 h-0
					24 h-71	24 h-27	24 h-0.08
1.5 ml Gel	2 h	N/A	N/A	N/A	30,394 (BLLOQ-1,162,921)	37,183 (BLLOQ-1,776,786)	138 (BLLOQ-726)
	4 h	35,081 (225–1,613,636)	10,181 (417–835,784)	19,250 (BLLOQ-1,200,000)	54,269 (BLLOQ-2,645,455)	152,548 (BLLOQ-6,993,750)	156 (BLLOQ-624)
	24 h	2390 (BLLOQ202.466)	12,200 (BLLOQ-546,825)	5377 (BLLOQ-23,226)	17,976 (BLLOQ-2,883,333)	56,977 (BLLOQ-830,952)	127 (BLLOQ-791)
	Ratio (measured/ EC_{50})	N/A	N/A	N/A	2 h-44.1	2 h-54	2 h-0.20
					4 h-78.8	4 h-221	4 h-0.23
					24 h-26.1	24 h-82.7	24 h-0.18

N/A not applicable, *BLLOQ* below lower limit of quantitation

[a] EC50 values of 1.0 ng/ml for IQP-0528 and 689 ng/ml for TFV were used to calculate measured drug/EC50 ratios

[b] P,M, and D refer to location of sample collection in relation to cervix. *P* proximal, *M* medial, *D* distal

Table 4

Drug concentrations in rectal tissues and rectal fluids from N = 6 female pigtailedmacaques following rectal application of 1 or 4 ml IQB4012 gel containing 1% wt/wt each of IQP-0528 and TFV^{+}

		IQP-0528 median drug concentration (min-max)		
		Rectal tissues (ng/g)		**Rectal fluids (ng/ml)**
1 ml Gel	2h	N/A		93,375 (2480–420,500)
	4h	9087 (BLLOQ[2]-15,150)		31,000 (545–873,500)
	24 h	127 *		2975 (815–22,900)
	Ratio (measured/EC_{50}[a])	N/A		2 h-93,375
				4 h-31,000
				24 h-2975
4 ml Gel	2h	N/A		106,500 (54,600–769,000)
	4h	1277 (BLLOQ-51,304)		53,775 (9570–930,500)
	24 h	566 (BLLOQ-2228)		7065 (608–43,750)
	Ratio (measured/EC_{50})	N/A		2 h-106,500
				4 h-53,775
				24 h-7065
		TFV/TFV-DP median drug concentration (min-max)		
		Rectal tissues (ng/g)		Rectal fluids (ng/ml)
		TFV	TFV-DP	
1 ml Gel	2h	N/A	N/A	3230 (BLLOQ-18,600)
	4h	811 (BLLOQ-1283)	176 (BLLOQ-207)	705 (BLLOQ-34,650)
	24 h	659 (BLLOQ-3586)	70 (BLLOQ-128)	132 *
	Ratio (Measured /EC_{50})	N/A	N/A	2 h-4.69
				4 h-1.02
				24 h-0.19
4 ml Gel	2h	N/A	N/A	5065 (BLLOQ-151,500)
	4h	393 (BLLOQ-11,464)	47 (BLLOQ-480)	864 (BLLOQ-147,000)
	24 h	1411 (BLLOQ-2648)	81 (BLLOQ-131)	277 (BLLOQ-816)
	Ratio (measured/EC_{50})	NA	NA	2 h-7.35

IQP-0528 median drug concentration (min-max)	
Rectal tissues (ng/g)	**Rectal fluids (ng/ml)**
	4 h-0.32
	24 h-0.12

N/A not applicable, *BLLOQ* below lower limit of quantitation

[a] EC50 values of 1.0 ng/ml for IQP-0528 and 689 ng/ml for TFV were used to calculate measured drug/EC50 ratios

* Drug detected in only one sample